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de Krom, Mariken; van der Schouw, Yvonne T.; Hendriks, Judith; Ophoff, Roel A.; van Gils, Carla H.; Stolk, Ronald P.; Grobbee, Diederick E.; Adan, Roger

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Brief Genetics Report

Common Genetic Variations in CCK, Leptin, and Leptin Receptor Genes Are Associated With Specific Human Eating Patterns

Mariken de Krom,[1] Yvonne T. van der Schouw,[2] Judith Hendriks,[1,3] Roel A. Ophoff,[3] Carla H. van Gils,[2] Ronald P. Stolk,[2,4] Diederick E. Grobbee,[2] and Roger Adan[1]

Obesity has a heritable component; however, the heterogeneity of obesity complicates dissection of its genetic background. In this study, we therefore focused on eating patterns as specific traits within obesity. These traits have a heritable component; genes associated with a specific eating pattern have not yet been reported at the population level. In this study, we determined whether genetic variations in cholecystokinin (CCK) and leptin genes underlie specific eating patterns. We selected obese individuals showing extreme snacking behavior or use of excessive portion sizes from a large population-based sample (n = 17,357) from the Prospect-EPIC (European Prospective Study into Cancer and Nutrition) study. Using allele-specific PCRs, we tested several single nucleotide polymorphisms in the candidate genes and performed haplotype analysis. Obese carriers of common allelic variations in leptin or the leptin receptor gene had an increased risk to display extreme snacking behavior. In contrast, obese carriers of common allelic variations in CCK had an increased risk to eating increased meal sizes. In conclusion, we identified common allelic variants specifically associated with distinctly different eating patterns, namely extreme snacking behavior or excessive portion size. *Diabetes* 56: 276–280, 2007

Obesity is an increasing problem in modern societies and a major risk factor for chronic diseases including diabetes, hypertension, and cardiovascular disease (1–3). Despite many genetic studies, only a small percentage of obesity cases can be directly explained by single gene mutations (4–7).

Different studies have shown a heritable component for eating behavior (8–12). Meal size and meal frequency are two eating patterns that show heritability (8–10). Only a few studies have been conducted to find genes underlying this heritability. Two studies have reported on genome-wide linkage screens: Steinle et al. (11) showed specific logarithm of odds scores for specific eating habits and Bouchard et al. (13) reported evidence of a specific candidate gene neuromedin B for eating behaviors and predisposition to obesity.

Two hormones, cholecystokinin (CCK) and leptin, have been implicated in the control of meal size and frequency in animal and human studies. Individuals with defects in the leptin gene have constant hunger and craving for food, which suggests a role for leptin in feelings of hunger in humans (14). However, mutations in leptin and its receptor explain only a very small proportion of the obese population. CCK is a satiety hormone. Administration of CCK in rats and humans results in a reduction of food intake (15–17). In contrast to leptin (receptor), no associations between obesity and the CCK gene have been described.

To determine whether common genetic variations in the CCK gene, the leptin gene, and its receptor contribute to abnormal eating habits in obese women, we performed a case-control study and tested these genes for association with meal size and meal frequency in a sample of individuals displaying at least one of these traits in an extreme form.

RESEARCH DESIGN AND METHODS

The study population was selected from the Prospect-European Prospective Study into Cancer and Nutrition (EPIC) study, a large population-based study that allowed the selection of obese individuals with specific eating patterns. The study is one of two Dutch components of the EPIC cohort, originally designed to investigate the role of nutrition factors in the occurrence of cancer (18). The cohort consists of 17,357 females aged 49–70 years at enrollment between 1993 and 1997 living in Utrecht, the Netherlands, or in the near vicinity with a Dutch ancestry. Detailed data on dietary habits, blood samples, BMI, and eating habits and physical activity (both based on a validated questionnaire) were present for all women (19,20) (Table 1). The cases were selected using the extreme discordant phenotype (EDP) approach (21) for an extreme meal size or meal frequency instead of on the broad phenotype of obesity; this selection results in a nine times–enhancement of statistical power (21).

Of the 17,357 women, a selection was made based on three criteria: BMI $\geq$33 kg/m^2; a score on snack behavior in the top 5th percentile, based on 11 questions regarding frequency of snack consumption; and a score in the top 5th percentile of food intake, based on 28 questions using color photographs to estimate portion size. As quality control measures, women with total energy intake <500 kcal/day or total energy intake >6,000 kcal/day were excluded. An exclusion criterion was the combination of high activity levels in the top 5th percentile, combined with high food intake, based on caloric intake.

From the [1]Department of Pharmacology and Anatomy, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Utrecht, the Netherlands; [2]The Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands; the [3]Division of Biomedical Genetics, University Medical Center Utrecht, Utrecht, the Netherlands; and the [4]Department of Epidemiology and Bioinformatics, University Medical Center Groningen, University of Groningen, the Netherlands.

Address correspondence and reprint requests to Dr. Mariken de Krom, Department of Pharmacology and Anatomy, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Universiteitsweg 100, 3584 CG Utrecht, Netherlands. E-mail: m.dekrom@med.uu.nl.

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CCK, cholecystokinin; EDP, extreme discordant phenotype; EPIC, European Prospective Study into Cancer and Nutrition; SNP, single nucleotide polymorphism.

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TABLE 1
Population characteristics of portion, snack, and control cases

Characteristic	Portion cases	Snack cases	Control cases
n	84	72	312
Age (years)	57 ± 6	58 ± 7	57 ± 6
Systolic blood pressure (mmHg)	143 ± 19	143 ± 18	132 ± 20
Diastolic blood pressure (mmHg)	85 ± 10	85 ± 11	78 ± 11
BMI (kg/m^2)	36 ± 3	36 ± 3	26 ± 4
Waist-hip ratio	0.84 ± 0.07	0.84 ± 0.06	0.79 ± 0.06
Never smokers	35 (2)	35 (49)	141 (45)
Premenopausal	9 (11)	6 (8)	31 (10)
Ever postmenopausal-hormone users	27 (33)	16 (22)	78 (25)

Data are means ± SD or *n* (%).

Beforehand, 1,450 women were excluded because of missing data on physical activity. We did not select highly active women, as they have a reason for high energy intake either through large meals or through frequent snacking. Based on activity level, 767 women were excluded before the selection of case and control subjects.

The selection resulted in 72 women who met the criteria BMI ≥33 kg/m^2 and scored in the top 5th percentile of meal size, 60 women who met the criteria BMI ≥33 kg/m^2 and whose snack behavior was in the top 5th percentile, and 3 women who met all three criteria for a total of 135 cases. A total of 287 control subjects were randomly selected from the total cohort. The BMI of this random selection was means ± SD 25.8 ± 1.46 kg/m^2. Within the random control group, there were women with snack (5%), meal size (2%), or both (1%) behaviors in the top 5th percentile but with a BMI <33 kg/m^2. The institutional review board of the University Medical Centre Utrecht, Utrecht, the Netherlands, approved the study and all participating subjects provided written consent.

Single nucleotide polymorphism selection and analysis. Haplotype-tagging single nucleotide polymorphisms (SNPs) were selected in the coding sequence and 100 kb up- and downstream of the genes using HapMap (Haploview 3.2) (available at http://www.gmap.net/perl/marker/marker) with r^2 >0.80 or Marker (22), which were available at the time of the study design. An SNP database search at the National Center for Biotechnology Information was conducted for the identification of nonsynonymous SNPs. This resulted in five haplotype-based SNPs for CCK, four SNPs for leptin, three haplotype-based and one coding SNP, and eight SNPs for the leptin receptor (seven were haplotype-based and one nonsynonymous SNP). All SNPs had a minor allele frequency of at least 15%.

All SNPs were genotyped using an allele-specific PCR. The PCR was performed using labeled primers with a 3′ locked nucleic acid, which is a modified SNP-binding nucleotide with a high binding affinity. Pooled PCR products determining different SNPs in one individual were separated by size on a 3700 capillary sequencer (ABI) and analyzed using the program Genemapper. This technique has been validated by comparing genotypes for multiple SNPs with those obtained from a Taqman system (ABI) with perfect agreement of results (data not shown).

Statistical analysis. The SNPs were tested for deviations from Hardy-Weinberg equilibrium using a Hardy-Weinberg *P* value cutoff of 0.01. Statistical analysis of the individual SNPs on genotype and allelic level was performed with the use of the two-sided Fisher's exact test. Haplotypes were determined using Haploview 3.2 (22). Differences in haplotype distribution were analyzed using a two-sided Fisher's exact test. The statistical significance level for all tests was set at $P < 0.05$. To correct for multiple testing using the permutation analysis function of UNPHASED, 100 permutations were performed.

TABLE 2
Genotype and allelic distributions of the CCK polymorphisms in different case populations compared with the control population

Polymorphism	Genotypes				Alleles		
rs6791019	TT	CT	CC	*P*	T	C	*P*
Total cases	56 (49)	45 (40)	13 (11)	0.001*	157 (69)	69 (31)	0.07
Portion cases	29 (43)	29 (43)	10 (14)	0.005*	87 (64)	49 (36)	0.009*
Snack cases	25 (58)	15 (35)	3 (7)	0.73	65 (76)	21 (24)	0.94
Controls	145 (56)	98 (38)	15 (6)		388 (75)	128 (25)	
rs7611677	CC	CT	TT	*P*	C	T	*P*
Total cases	89 (76)	25 (21)	3 (3)	0.07	203 (87)	31 (13)	0.03*
Portion cases	44 (68)	19 (29)	2 (3)	0.005*	107 (82)	23 (18)	0.002*
Snack cases	42 (86)	6 (12)	1 (2)	0.47	90 (92)	8 (8)	0.99
Controls	201 (84)	35 (15)	2 (1)		437 (92)	39 (8)	
rs6809785	CC	CG	GG	*P*	C	G	*P*
Total cases	74 (76)	23 (24)	0	0.02*	171 (88)	23 (12)	0.05
Portion cases	38 (70)	16 (30)	0	0.001*	92 (85)	16 (15)	0.01*
Snack cases	33 (83)	7 (17)	0	0.42	73 (91)	7 (9)	0.62
Controls	198 (86)	29 (13)	2 (1)		425 (93)	33 (7)	
rs11129946	GG	GT	TT	*P*	G	T	*P*
Total cases	53 (59)	34 (37)	4 (4)	0.03*	140 (77)	42 (23)	0.42
Portion cases	34 (64)	18 (34)	1 (2)	0.02*	86 (81)	20 (19)	0.11
Snack cases	18 (51)	14 (40)	3 (9)	0.71	50 (71)	20 (29)	0.66
Controls	144 (58)	77 (31)	26 (11)		365 (74)	129 (26)	
rs6801844	GG	GA	AA	*P*	G	A	*P*
Total cases	50 (52)	39 (41)	7 (7)	0.23	139 (72)	53 (28)	0.29
Portion cases	24 (45)	24 (45)	5 (10)	0.09	72 (68)	34 (32)	0.07
Snack cases	24 (60)	14 (35)	2 (5)	0.72	62 (78)	18 (23)	0.82
Controls	136 (59)	79 (34)	15 (7)		351 (76)	109 (24)	

Data are *n* (%). For some sequences, only a 70–75% success rate could be reached due to a less efficient allele-specific PCR. **P* values are significant.

TABLE 3
Genotype and allelic distributions of the leptin receptor and leptin gene polymorphisms in the different case populations compared with the control population

Polymorphism	Genotypes				Alleles		
rs2025804	AA	AG	GG	*P*	A	G	*P*
Total cases	47 (41)	51 (44)	17 (15)	0.21	145 (63)	85 (37)	0.37
Portion cases	28 (43)	30 (46)	7 (11)	0.76	86 (66)	44 (34)	0.78
Snack cases	17 (36)	20 (43)	10 (21)	0.02*	54 (57)	40 (43)	0.06
Controls	111 (44)	114 (46)	24 (10)		336 (67)	162 (33)	
rs1782754	GG	GA	AA	*P*	G	A	*P*
Total cases	56 (49)	47 (42)	10 (9)	0.68	159 (70)	67 (30)	0.85
Portion cases	33 (51)	28 (43)	4 (6)	0.76	94 (72)	36 (28)	0.78
Snack cases	21 (47)	18 (40)	6 (13)	0.77	60 (67)	30 (33)	0.43
Controls	88 (51)	68 (40)	16 (9)		244 (71)	100 (29)	
rs7529650	GG	GA	AA	*P*	G	A	*P*
Total cases	46 (40)	46 (40)	24 (20)	0.05	138 (59)	94 (41)	0.47
Portion cases	25 (38)	29 (45)	11 (17)	0.39	79 (61)	51 (39)	0.44
Snack cases	19 (40)	16 (33)	13 (27)	0.11	54 (56)	42 (44)	0.91
Controls	63 (32)	96 (49)	36 (19)		222 (57)	168 (43)	
rs1171265	AA	AG	GG	*P*	A	G	*P*
Total cases	44 (46)	41 (43)	11 (11)	0.14	129 (67)	63 (33)	0.17
Portion cases	23 (43)	24 (44)	7 (13)	0.37	70 (65)	38 (35)	0.47
Snack cases	19 (49)	16 (41)	4 (10)	0.17	54 (69)	24 (31)	0.17
Controls	86 (41)	82 (40)	40 (19)		254 (61)	162 (39)	
rs1137100	AA	AG	GG	*P*	A	G	*P*
Total cases	45 (49)	39 (42)	0 (9)	0.23	129 (70)	55 (30)	0.58
Portion cases	25 (52)	20 (42)	3 (6)	0.46	70 (73)	26 (27)	0.97
Snack cases	19 (45)	18 (43)	5 (12)	0.25	56 (67)	28 (33)	0.26
Controls	123 (55)	77 (35)	22 (10)		323 (73)	121 (27)	
rs1137101	GG	GA	AA	*P*	G	A	*P*
Total cases	31 (28)	47 (42)	34 (30)	0.12	109 (49)	115 (51)	0.99
Portion cases	14 (23)	25 (41)	22 (36)	0.46	53 (43)	69 (57)	0.97
Snack cases	16 (33)	20 (42)	12 (25)	0.09	52 (54)	44 (46)	0.33
Controls	52 (22)	122 (53)	58 (25)		226 (49)	238 (51)	
rs6673324	AA	AG	GG	*P*	A	G	*P*
Total cases	25 (27)	49 (53)	18 (20)	0.05	99 (54)	85 (46)	0.73
Portion cases	12 (23)	29 (57)	10 (20)	0.66	53 (52)	49 (48)	0.49
Snack cases	12 (31)	19 (49)	8 (20)	0.36	43 (55)	35 (45)	0.92
Controls	72 (36)	79 (40)	49 (24)		223 (56)	177 (44)	
rs1327116	AA	AC	CC	*P*	A	C	*P*
Total cases	57 (51)	46 (41)	9 (8)	0.92	160 (71)	64 (29)	0.99
Portion cases	31 (50)	27 (44)	4 (6)	0.57	89 (72)	35 (28)	0.99
Snack cases	24 (51)	18 (38)	5 (11)	0.96	66 (70)	28 (30)	0.76
Controls	133 (52)	98 (39)	23 (9)		366 (72)	144 (28)	
rs791607	TT	CT	CC	*P*	T	C	*P*
Total cases	90 (89)	11 (11)	0	0.10	191 (95)	11 (5)	0.08
Portion cases	50 (85)	9 (15)	0	0.57	109 (92)	9 (8)	0.54
Snack cases	39 (98)	1 (2)	0	0.01*	79 (99)	1 (1)	0.61
Controls	155 (81)	34 (18)	1 (1)		344 (91)	36 (9)	
rs4577902	TT	CT	CC	*P*	T	C	*P*
Total cases	75 (73)	27 (26)	1 (1)	0.32	177 (86)	29 (14)	0.42
Portion cases	42 (72)	15 (26)	1 (2)	0.32	99 (85)	17 (15)	0.30
Snack cases	31 (74)	11 (26)	0	0.006*	73 (87)	11 (13)	0.58
Controls	193 (79)	49 (20)	3 (1)		435 (89)	55 (11)	
rs2060736	CC	CG	GG	*P*	C	G	*P*
Total cases	44 (72)	16 (26)	1 (2)	0.36	104 (85)	18 (15)	0.71
Portion cases	17 (65)	9 (35)	0	0.18	43 (83)	9 (17)	0.83
Snack cases	14 (74)	5 (26)	0	0.22	33 (87)	5 (13)	0.63
Controls	138 (72)	46 (24)	8 (4)		322 (84)	62 (16)	
rs4731413	GG	GA	AA	*P*	G	A	*P*
Total cases	59 (65)	26 (29)	5 (6)	0.1	144 (80)	36 (20)	0.44
Portion cases	30 (65)	14 (31)	2 (4)	0.35	74 (80)	18 (20)	0.62
Snack cases	27 (64)	12 (29)	3 (7)	0.07	66 (79)	18 (21)	0.37
Controls	164 (67)	75 (31)	5 (2)		403 (83)	85 (17)	

**P* values are significant. Numbers in parenthesis are the percentages of the genotypes present in the different groups. For some sequences, only a 70–85% success rate could be reached due to a less efficient allele-specific PCR.

TABLE 4
Haplotype frequencies in the case populations compared with the control population

Haplotype identification	Haplotype sequence	Total cases	*P*	Portion	*P*	Snack	*P*	Control
CCK_H1	CCG**T**	0.71 (153)	0.9	0.67 (82)	0.4	0.77 (71)	0.21	0.71 (268)
CCK_H2	CCAA	0.14 (30)	0.53	0.15 (18)	0.77	0.13 (12)	0.48	0.16 (60)
CCK_H3	**GT**AA	0.13 (28)	0.23	0.17 (21)	0.02*	0.08 (7)	0.51	0.1 (37)
CCK_other		—	—	—	—	—	—	0.03 (11)
Lep_H1	GT**C**	0.62 (105)	0.42	0.60 (52)	0.71	0.68 (53)	0.03*	0.58 (252)
Lep_H2	ATC	0.21 (26)	0.71	0.19 (16)	0.37	0.13 (10)	0.77	0.15 (64)
Lep_H3	GT**G**	0.04 (7)	0.002*	0.06 (5)	0.06	0.03 (2)	0.01*	0.13 (56)
Lep_H4	GCC	0.13 (22)	0.94	0.15 (13)	0.55	0.12 (9)	0.89	0.13 (55)
Lep_other		—	—	—	—	—	—	0.01 (5)

Data are frequency (n). *P values are significant.

RESULTS

We genotyped 17 SNPs located in the 3′ untranslated region or coding region of CCK ($n = 5$), leptin ($n = 4$), and the leptin receptor ($n = 8$) in the total sample ($n = 422$). All SNPs showed the expected genotype proportions when tested for Hardy-Weinberg equilibrium.

The case subjects were categorized according to extreme snack behavior ($n = 60$) and meal size ($n = 72$). The genotype and allelic distributions of the 17 SNPs were compared between the two selection groups and the random control sample ($n = 287$); global P values were determined, and haplotypes were subsequently established.

Four of the five tested CCK SNPs showed a specific association signal with extreme meal size ($P = 0.005$, $P = 0.00$, $P = 0.001$, and $P = 0.02$) but not with extreme snack behavior (Table 2). One of eight SNPs of the leptin receptor and two of four SNPs of leptin were associated with extreme snack behavior but not with meal size ($P = 0.02$, $P = 0.01$, and $P = 0.006$) (Table 3). Furthermore, (corrected) global P values, derived from single SNPs, show significant associations (CCK $P = 0.029$, leptin receptor $P = 0.009$, and leptin $P = 0.0198$) with meal size and snacking.

Using Haploview 3.2 (22) we constructed haplotypes to determine whether specific haplotypes underlie the associations found. Haplotypes were determined for CCK, CCK_H1–H5 (rs6809785, rs7611677, rs6801844, and rs6791019) and leptin, Lep_H1–H7 (rs4577902, rs2060736, and rs4731413). A very high D′ (close to 1) (Tables 4 and 5) was detected between the haplotype SNPs within both CCK and leptin. This indicates that the haplotype data do reflect the findings of the individual SNPs (i.e., significantly associated). No haplotypes could be determined for the leptin receptor. (Tables 4 and 5) using Haploview 3.2.

TABLE 5
Physical genomic distance and D′ between haplotype SNPs

Gene	SNP	Genomic location	D′
CCK	1:rs6809785	42245074	SNP 1–2: 0.984
	2:rs7611677	42251928	SNP 1–3: 0.924
	3:rs6801844	42254089	SNP 1–4: 1
	4:rs6791019	42254957	SNP 2–3: 0.941
			SNP 2–4: 1
			SNP 3–4: 0.945
Leptin	1:rs4731413	127430083	SNP 1–2: 0.999
	2:rs4577902	127436760	SNP 1–3: 1
	3:rs2060736	127442525	SNP 2–3: 1

CCK_H3 showed an increase in frequency in extreme portion eaters compared with control subjects, an increase from 10% ($n = 37$) to 17% ($n = 21$) ($P = 0.022$), suggesting that H3 is a risk haplotype for an increased meal size. The three SNPs determining this haplotype also individually showed an association with this phenotype.

In agreement with the observed association for the allelic variations for the leptin gene, the SNPs also showed at the haplotype level a specific association with extreme snacking. The most frequent haplotype (Lep_H1) was observed in the extreme snack group (58% [$n = 252$] vs. 68% [$n = 53$], $P = 0.03$), while a clear loss of Lep_H3 was seen (13% [$n = 56$] to 3% [$n = 2$], $P = 0.01$). These data suggest that there is a protective (Lep_H3) and a risk (Lep_H1) haplotype for extreme snack behavior.

We also assessed the SNPs in the total case group. Three of the individually tested SNPs, rs6791019, rs6809785 and rs11129946, of CCK showed an association ($P = 0.001$, $P = 0.02$, $P = 0.03$, respectively, Table 2).

Finally we checked the haplotype distribution within the random control group. Within the control group 5% ($n = 15$) and 2% ($n = 7$) displayed extreme snacking or meal size without a BMI ≥33 kg/m^2. The associated haplotypes found in the obese group were borderline associated in the extreme snackers (Lep_H1, $P = 0.05$) and not in the portion eaters (CCK_H3, $P = 0.74$) in the control group. The very small number of women included in these groups probably caused this. Of the total random control group, 13.5% had a BMI ≥33 kg/m^2, without displaying the specific eating patterns. The allelic and haplotype distributions of the CCK, leptin, and leptin receptor genes in the group with BMI ≥33 kg/m^2 did not differ from the distributions found in the rest of the control group.

DISCUSSION

In this study, we associated SNPs and haplotypes in the CCK gene with large meal size and in the leptin genes with high meal frequencies, two specific eating patterns within the broad phenotype of obesity.

We took an alternative approach to previous research addressing the genetic basis of obesity. Using the EDP approach we selected cases based on the extreme expression of specific traits of food intake and show that this mode of selection is useful in determining common genetic variation underlying a specific trait within a broad phenotype. Interestingly, the selected population fell into two almost nonoverlapping groups, which for a significant portion was explained by genetic variation at the three

genes that were genotyped. This may not be surprising since extreme snacking may decrease meal size.

As far as we know, no association studies have been reported on the role of the CCK gene in obesity, satiety, or meal size. In this study we found associations between CCK polymorphisms/haplotypes and excessive meal size, which probably contribute to problems with satiety signaling in these carriers.

Some genetic studies have been conducted on the role of leptin genes in obesity (5,14,23,24). Only a small number of rare variants have been found explaining only a minority of the obese cases. Our findings suggest a specified role of the leptin genes in obesity, namely in extreme meal frequencies in the population sample.

The usefulness of the EDP approach is illustrated by the different frequencies of the observed Lep_H1 haplotypes in the extreme cases (68%) and the overall cases (62%) versus the control sample (58%). It is remarkable that such a common haplotype was found to be associated with extreme snacking. Further study is required to investigate the genetic history of this haplotype and its possible biological relevance for obesity.

Based on the haplotype and genotype associations, we could determine that obese carriers of variants in the CCK gene have an increased risk of eating large portion sizes, with a 60% increased risk for carriers of the CCK_H3 haplotype and that obese carriers of variants in the leptin genes have an increased risk if displaying extreme snack behavior, with a 20% increased risk for Lep _H1 haplotype carriers. However, given the high frequency of this allele in the population, the population attributable risk is very high.

Further studies are necessary to confirm our findings and to examine the effect of the genetic variants in order to be able to evaluate the general risk in the population. It will be interesting to see to what extent the found risk alleles and haplotypes contribute to eating patterns in the general population.

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